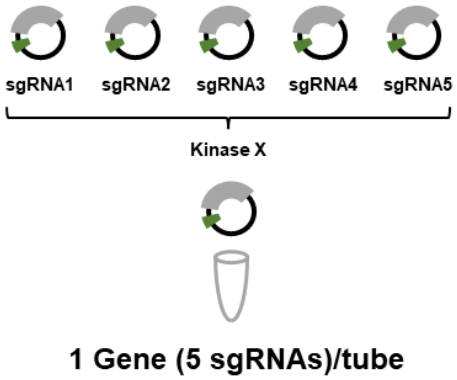
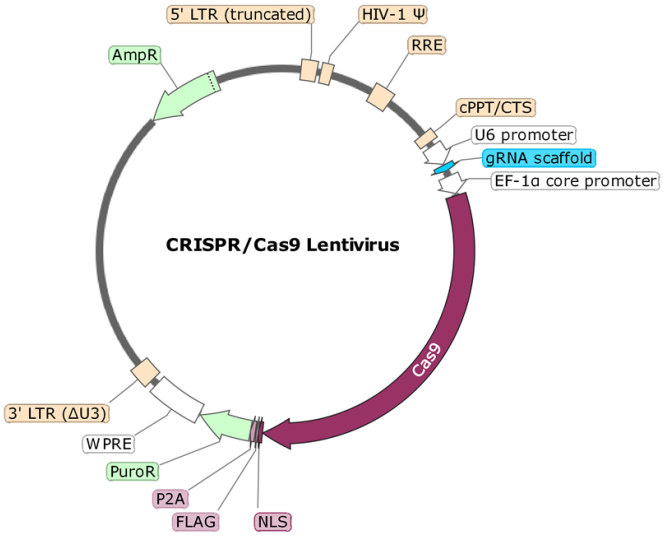
**Description**

The CRISPR/Cas9 Kinase Knockout Lentivirus Library (Array Format) targets 619 human kinases and pseudo-kinases. A table containing the complete list of kinases and pseudo-kinases can be downloaded as an excel file from our website. The Array consists of a series of vials, with each vial containing a mixture of integrating CRISPR/Cas9 lentiviral particles targeting 5 sgRNAs for a specific gene (1 vial per gene, 5 sgRNAs per gene). The Array also includes a total of 150 control sgRNAs that do not target any gene (combined into 30 vials containing 5 control sgRNAs per vial). Thus, the Array contains a total of 649 vials and 3,245 sgRNAs.




The lentiviruses are replication incompetent, VSV-G pseudotyped lentiviral particles ready to infect almost all types of mammalian cells, including primary and non-dividing cells. The SIN (self-inactivation) lentiviral backbone contains the Cas9 gene (*Streptococcus pyogenes* CRISPR associated protein 9) driven by an EF1A promoter, an sgRNA driven by a U6 promoter, and a puromycin selection marker.



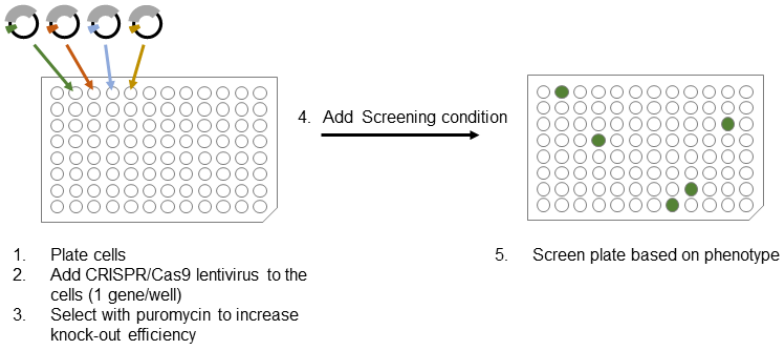

*Schematic of the lentivector used to generate each kinase CRISPR/Cas9 Lentivirus.*

The lentiviruses integrate randomly into the cellular genome to express both Cas9 and the sgRNAs. Because the lentiviruses contain Cas9, they can be used in any target cell regardless of whether the cells already express Cas9. Puromycin selection ensures high expression of both Cas9 and the sgRNAs. Knockout efficiencies will depend on the cell type and the gene of interest. Stable CRISPR/Cas9 knockout cell lines can also be generated following limiting dilution.

The library is delivered with a User Manual booklet.

**Applications**

Many applications require a simple phenotypic readout and are therefore amenable to high throughput screening.




1. **Viability Screening**
   Perform a knockout screen in 96-well plates and measure the resulting cell viability or cell proliferation to determine which gene knockout causes the cells to die or to stop growing. This type of screen identifies genes involved in cell survival or genes required for cell proliferation.

2. **Synthetic Lethality Screening**
   Perform a knockout screen in 96-well plates, add compounds of interest to the knockout cells, and measure the resulting cell viability to identify genes which, when depleted, cause the target cells to become sensitive to the compounds being tested.

3. **Drug Resistance Screening**
   Perform a knockout screen in 96-well plates and add a drug of interest. Then, measure the cell survival or cell proliferation to determine which genes, when knocked out, enable the cells to become resistant to the drug. Identification of these genes may provide insight into pathways of drug resistance.

4. **Transcriptional Activation (Cell reporter system)**
   Perform a knockout screen in 96-well plates utilizing cells expressing a luciferase or fluorescent reporter under the control of a specific promoter sequence. Identify genes which, when knocked out, induce either the activation or inactivation of the signaling pathway being probed by the reporter.

5. **Cell Signaling (Protein expression or phosphorylation)**
   Perform a knockout screen in 96-well plates to determine which kinase is involved in either the regulation of a specific phosphorylation event or that controls the expression levels of a protein of interest. Readouts may include FACS, Western blots, etc.

**Formulation**

The lentivirus particles are produced from HEK293T cells. They are supplied in cell culture medium containing 90% DMEM + 10% FBS.

**Titer**

Each of the 649 vials come with 200 µl of lentivirus at a titer ≥1 x $10^7$ TU/ml. The titer varies slightly with each lot; the exact titer is provided with each shipment. Each lentivirus was individually titered to ensure representation across the entire library. Cell titers were determined by measuring the lentiviral structural protein p24 (using Lenti-X GoStix Plus by Takara).

**Storage**

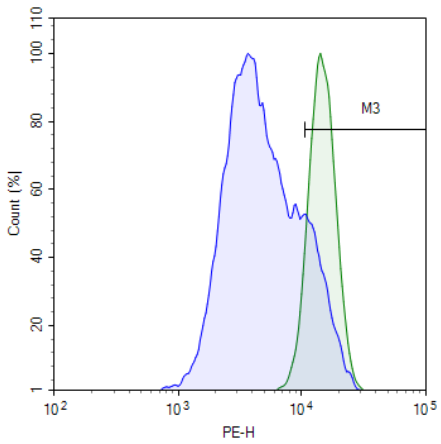
Lentiviruses are shipped with dry ice. For long-term storage, it is recommended to store the lentiviruses at -80°C. Avoid repeated freeze/thaw cycles. Titers can drop significantly with each freeze/thaw cycle.

**Biosafety**

The lentiviruses are produced with the SIN (self-inactivation) lentivector which ensures self-inactivation of the lentiviral construct after transduction and after integration into the genomic DNA of the target cells. None of the HIV genes (gag, pol, rev) will be expressed in the transduced cells, as they are expressed from packaging plasmids lacking the packing signal and are not present in the lentivirus particle. Although the lentiviruses are replication-incompetent, they require the use of a Biosafety Level 2 facility. BPS Bioscience recommends following all local federal, state, and institutional regulations and using all appropriate safety precautions.

**Validation Data**

**A.**

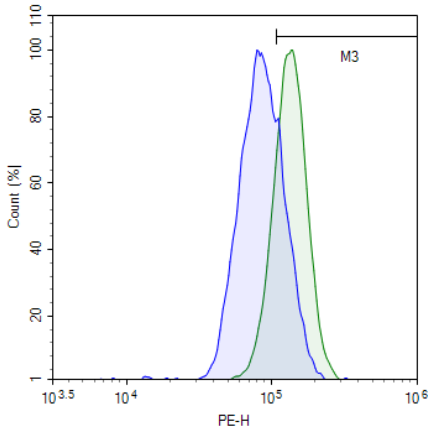



**B.**

| Sample | Gate | % of Cell Population |
|---|---|---|
| Jurkat Parental cells | M3 | 90.57% |
| Jurkat cells transduced with ABL1 CRISPR/Cas9 lentivirus | M3 | 16.40% |

*Figure 1. Knockout of ABL1 in Jurkat cells.*
**A.** Jurkat cells were transduced via spinoculation with CRISPR/Cas9 lentivirus targeting ABL1 (Gene 367), corresponding to an MOI of approximately 1. Cells were enriched using puromycin selection for 24-48 hours, stained with rabbit anti-ABL1 (Abcam #ab254341) and PE-conjugated anti-rabbit secondary antibodies (BioLegend #406421), and analyzed by flow cytometry. Parental Jurkat cells are shown in green, and the transduced cells are shown in blue. **B.** Percentages of cell populations captured in Gate M3.

**A.**

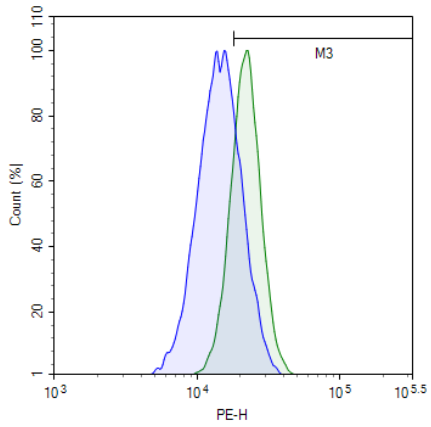



**B.**

| Sample | Gate | % of Cell Population |
|---|---|---|
| Jurkat Parental cells | M3 | 80.25% |
| Jurkat cells transduced with ZAP70 CRISPR/Cas9 lentivirus | M3 | 26.82% |

*Figure 2. Knockout of ZAP70 in Jurkat cells.*
**A.** Jurkat cells were transduced via spinoculation with CRISPR/Cas9 lentivirus targeting ZAP70 (Gene 442), corresponding to an MOI of approximately 1. Cells were enriched using puromycin selection for 24-48 hours, stained with mouse anti-ZAP70 (BioLegend #691202) and PE-conjugated anti-mouse secondary antibodies (BioLegend #405307), and analyzed by flow cytometry. Parental Jurkat cells are shown in green, and the transduced cells are shown in blue. **B.** Percentages of cell populations captured in Gate M3.

**A.**




**B.**

| Sample | Gate | % of Cell Population |
|---|---|---|
| Jurkat Parental cells | M3 | 79.12% |
| Jurkat cells transduced with ITK CRISPR/Cas9 lentivirus | M3 | 26.30% |

*Figure 3. Knockout of ITK in Jurkat cells.*
**A.** Jurkat cells were transduced via spinoculation with CRISPR/Cas9 lentivirus targeting ITK (Gene 445), corresponding to an MOI of approximately 1. Cells were enriched using puromycin selection for 24-48 hours, stained with mouse anti-ITK (BioLegend #687302) and PE-conjugated anti-mouse secondary antibodies (BioLegend #405307), and analyzed by flow cytometry. Parental Jurkat cells are shown in green, and the transduced cells are shown in blue. **B.** Percentages of cell populations captured in Gate M3.

**Troubleshooting Guide**
Visit bpsbioscience.com/lentivirus-faq. For all further questions, please email support@bpsbioscience.com.

**Disclaimer and Limitations**
The knock-out efficiency of CRISPR/Cas9 gene editing can be influenced by various factors such as the gene of interest, experimental conditions, cell type, and guide RNA design. Therefore, performance cannot be guaranteed.

**Notes**
The CRISPR/CAS9 technology is covered under numerous patents, including U.S. Patent Nos. 8,697,359 and 8,771,945, as well as corresponding foreign patents applications, and patent rights.

**Related Products**

| *Products* | *Catalog #* | *Size* |
|---|---|---|
| Kinase (Human) CRISPR/Cas9 Lentivirus – Individual Kinase | 78488 | 2 x 200 µl |
| Custom panels | Custom order | various |

**Custom Panels**
Build your own Kinase Knockout Lentivirus Panel directly on our website. Our customer service will provide you a quote for any build. Visit webpage.